REMARKS

A. Status of Claims.

Claims 1, 3, 8 and 9 are currently pending. Claim 1 is amended and Claims 2 and 4 through 7 are cancelled.

B. Support for Claim Amendments.

Support for the phrase, "causing splitting, loss or deletion of a chromosome in a yeast" in amended Claim 1 is found throughout the application, but particularly on pages 12 through 14 as well as Figures 3, 4, 6 and 8.

Support for the phrase, "simultaneously introducing the chromosome splitting vectors (1) and (2) into a yeast" in amended Claim 1 (according to one embodiment of using the two vectors in tandem) is found throughout the application, but particularly on pages 12 through 13 and Figures 3 and 4 as well as the examples provided in Figures 6 through 10 and associated text.

Support for the phrase, "selecting yeast on the basis of marker gene expression" in amended Claim 1 is found throughout the application and is inherent to the use of selection makers. However, particular support is found on pages 14, paragraph [61]; page 19, paragraph [83]; and page 22, paragraph [95].

Finally, support for the phrase, "wherein the chromosome splitting vectors (1) and (2) are obtained directly by a PCR reaction, wherein at least one primer used in each PCR reaction includes the $(C_4A_2)_n$ sequence" is found throughout the application, but particularly on pages 12 through 14 and Figures 3 and 5 as well as the examples provided in Figures 6 through 10 and associated text. Support for the phrase, "wherein at least one primer used in each PCR reaction includes the $(C_4A_2)_n$ sequence" is further found on pages 11 and 12 of the application.

C. Rejections under Section 103(a)

Claims 1 through 7 are rejected under 35 U.S.C. 103(a) as being allegedly unpatentable over Widianto, *et al.* (J. Fragmentation and Bio Engineering, 82(3):199 through

204, 1996, *ref. of record on PTO 1449)* (**Widianto**) and Ascenzioni, *et al.* (PLASMID 23: 16 through 26, 1990, *ref. of record on PTO 1449)* (**Ascenzioni**). For purposes of this response, it is assumed that the Claims are rejected over **Widianto** *in view of* **Ascenzioni**. However, for reasons explained below, Applicants assert that the Office Action has failed to establish a *prima facie* case of obviousness since (i) the combined teachings of the cited references do not teach all of the elements and features of the claimed invention and (ii) a person of ordinary skill would not have reason to combine those references in the manner claimed.

I. *The combined teachings of the cited references do not teach all of the elements and features of the claimed invention.*

Widianto describes a method of splitting a yeast chromosome using a plasmid vector that is cut by a restriction enzyme to generate a linear vector having an order consisting of (i) a first portion of a target sequence, (ii) two opposing telomeres, (iii) a centromere, (iv) a marker, and (v) a second portion of a target sequence, where the first and second portions of the target sequence refer to contiguous sequences at a single locus within a yeast chromosome.[1] The method of producing the linear splitting vector in **Widianto** is essentially the same as the conventional method described in Figure 17 of the application, and the mechanism of splitting the chromosome in **Widianto** is essentially the same as the conventional method described in Figure 15 of the application. Upon comparison of the method in **Widianto** with the claimed invention, it becomes apparent that that the present claims provide a patentably distinct method of causing splitting, loss or deletion of a yeast chromosome through a distinct mechanism that has important advantages over the conventional approach in **Widianto**.

One key element or feature of the presently claimed invention (that is absent from **Widianto**) is the ability to use PCR to directly prepare the splitting vectors (1) and (2) in a form that is ready for immediate use. Under a conventional approach, such a PCR method to prepare a splitting vector(s) would have needed to overcome the difficulty of amplifying repetitive telomeric sequences, such as the *Tetrahymena* (Tr) sequence used in **Widianto**.[2] However, the present invention as claimed overcomes this difficulty in part by incorporating a short minimal telomere sequence $(C_4A_2)_n$, wherein n is independently an integer of 6 to 10, (as well as target sequences) into the PCR primers themselves. As a result, splitting vectors

[1] See, Widianto, Figures 1 and 2.
[2] See, Application, p. 4, paragraphs [11] through [13] and Figure 16.

(1) and (2), including all of the elements necessary for causing splitting, loss or deletion of a yeast chromosome, can be generated through a single PCR reaction step using a template for the centromere or marker gene sequences. Therefore, the particular design and outcome of the claimed method of causing splitting, loss or deletion of a yeast chromosome is largely determined by the PCR conditions and exact primer sequences chosen.

In contrast, **Widianto** only describes use of a single linear vector generated through a series of cloning steps and restriction enzyme digestions. Nowhere does **Widianto** teach or even suggest the use of PCR to directly generate the two splitting vectors claimed by incorporating sequence elements into the PCR primer themselves. In fact, it would be challenging at best to generate the linear vector in **Widianto** by PCR due to the difficulty of amplifying long repetitive *Tetrahymena* (Tr) telomeric sequences as indicated, for example, by the experiments in Figure 18 of the present application.[3] In contrast to **Widianto**, the claimed invention is specifically designed to overcome this difficulty by incorporating a short $(C_4A_2)_n$ telomere sequence into the primers used for PCR.

Another major difference between **Widianto** and the presently claimed invention is the order and relationship of sequence elements within the vector(s) used. First, the method in **Widianto** uses only a single linear vector generated by a restriction enzyme digestion within the target sequence of the plasmid (pDW10 or pDW18).[4] In contrast, amended claim 1 provides for two separate and structurally distinct vectors produced by PCR (*i.e.*, splitting vectors (1) and (2)) that are introduced simultaneously into the yeast cell to allow the two splitting vectors to work in tandem. Second, once the plasmid vector in **Widianto** is cut within the target sequence to yield a single linear fragment, the order of elements is (a) a first portion of the target sequence, (b) two opposing telomeres, (c) a centromere, (d) a marker, and (e) a second portion of the target sequence. In contrast, the order of sequence elements for each of the splitting vectors (1) and (2) generated by PCR of the presently claimed invention is as follows:

1. A linear chromosome splitting vector (1) in an order consisting of a **target sequence** (a), a **marker gene sequence**, and **$(C_4A_2)_n$ sequence** (x); and
2. A linear chromosome splitting vector (2) in an order consisting of a **target sequence** (b), a **centromere sequence** of a yeast chromosome, and **$(C_4A_2)_n$ sequence** (y).

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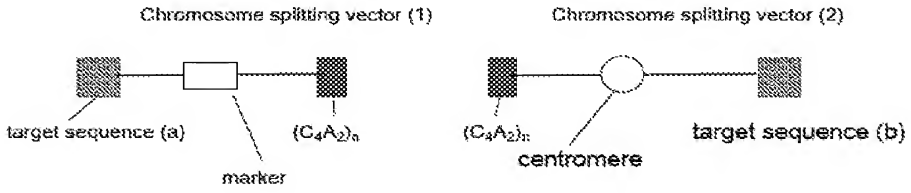
[3] See, Application, p. 4, paragraphs [11] and [12]; See also, **Widianto**, Materials and Methods on p. 199 ("The stuffer DNA between the inverted [Tr] repeats is necessary for propagation of pCSV1 in *E. Coli*").
[4] See, **Widianto**, Figures 1 and 2.

Thus, in contrast to the single vector in **Widianto**, not only does amended claim 1 describe (i) the use of two separate splitting vectors acting in tandem, it also (ii) segregates functionally distinct elements between the two vectors and (iii) places these elements in a different order along each of the two vectors. Compare the order and arrangement of the following two splitting vectors as claimed with the single linear vector in Figure 2 of **Widianto**:



A significant advantage of the present claims over the method in **Widianto** is that the target sequence in **Widianto** is limited to a single locus within the yeast genome since, as shown, the two arms or portions of the target sequence are produced simply by cutting the plasmid at a restriction site within an intact and contiguous target sequence corresponding to a single location in the yeast genome.[5] In contrast, however, each of the target sequences (a) and (b) of splitting vectors (1) and (2), respectively, of the presently claimed invention could potentially correspond to either a single location or two different locations of a yeast chromosome. As a result, not only do the placement and segregation of sequence elements between the splitting vectors (1) and (2) give rise to a different mechanism of action compared to the method in **Widianto**, such arrangement of sequence elements also has a profound impact on the versatility and number of outcomes that can be achieved by using the PCR-based method of the claimed invention, some of which are simply not possible with the method described in **Widianto**.

To illustrate this point, according to some embodiments, the target sequences (a) and (b) on chromosome splitting vectors (1) and (2), respectively, could correspond to nearby or contiguous sequences such that the yeast chromosome is split at a location corresponding to the target sequence.[6] Alternatively, an intervening portion of a yeast chromosome could also

[5] See, **Widianto**, Figures 1 and 2.
[6] See, Application, p. 19, paragraph [82] and Figure 6. ("Type A" in Figure 6A results in splitting of the yeast chromosome at the location corresponding to the target sequences (a) and (b). A similar outcome occurs at such location along the yeast chromosome by inverting the relative placement of the two target sequences (a) and (b)

be deleted by selecting target sequences (a) and (b) of chromosome splitting vectors (1) and (2), respectively, such that they correspond to positions that are distanced apart from each other along the yeast chromosome.[7] Nowhere does **Widianto** describe the ability of using target sequences that correspond to genomic sequences that are separated at a distance from one another. Since **Widianto** relies on a different mechanism and arrangement of elements compared to the method as claimed, it is not certain whether using such non-contiguous target sequences would even function in a yeast cell according to the method described in **Widianto** due to the different orientation of sequence elements as well as their inter-connectivity as part of a single vector.

Finally, the Office Action admits that **Widianto** does not teach the use of a synthetic C_4A_2 telomere sequence, but cites **Ascenzioni** as teaching the use of such C_4A_2 telomere sequences. However, **Ascenzioni** merely describes the use of synthetic C_4A_2 telomere sequences in a yeast artificial chromosome (YAC) to study whether ARS sequences are required for telomere function. Nowhere does **Ascenzioni** describe the use of vector(s) to cause modification, splitting, loss, or deletion of an existing yeast chromosome on the basis of a target sequence(s) corresponding to a region(s) of a yeast chromosome. Instead, **Ascenzioni** merely describes the introduction of a new synthetic chromosome into yeast for purposes of experimentation. Therefore, **Ascenzioni** does not teach or suggest any of the elements or features of the claimed invention that are missing from **Widianto**.

In view of the foregoing, **Widianto** does not teach or suggest the use of two separate chromosome splitting vectors generated by PCR using primers that incorporate the $(C_4A_2)_n$ sequence and have the particular order and arrangement of sequence elements as claimed. Furthermore, **Ascenzioni** does not cure these deficiencies in **Widianto**. Even assuming that the linear vector in **Widianto** were modified to replace the *Tetrahymena* (Tr) sequence with a $(C_4A_2)_n$ telomere sequence in **Ascenzioni**, such combination would still not teach or suggest all of the elements and features of the claimed invention for the reasons described above. Therefore, since the combined teachings of the cited references do not teach all of the

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as in "Type B" in Figure 6B as long as the target sequences remain in close proximity with the minor difference being that the target sequences are not introduced into the yeast genome under the Type B scenario.).

[7] See, Application, p. 19, paragraph [82] and Figure 6. (If the relative positions of the two target sequences (a) and (b) on each of the splitting vectors are not only inverted as in the Type B scenario, but are also positioned at a distance apart from each other along the yeast chromosome, then intervening sequences will also be deleted.).

elements or features of the claimed invention, Applicants respectfully assert that the Office Action has failed to establish a *prima facie* case of obviousness under §103(a).[8]

II. *A person of ordinary skill would not have reason to combine the cited references in the manner claimed.*

Not only do the cited references not teach or suggest all of the elements and features of the claimed invention, a person of ordinary skill would not have reason to combine the teachings of **Widianto** and **Ascenzioni** in the manner claimed.[9] **Widianto** teaches a method for splitting an existing yeast chromosome using a single linear vector generated by a sequence of cloning steps and restriction enzyme digestions. On the other hand, **Ascenzioni** describes the introduction of a yeast artificial chromosome (YAC) containing a C_4A_2 telomere sequence into yeast. Therefore, a person of ordinary skill would not have reason to combine the cited references since **Ascenzioni** is directed toward a different purpose and not toward modifying an existing chromosome in yeast as in **Widianto**.

The Office Action further cites **Ascenzioni** for teaching the use of synthetic C_4A_2 telomere sequences with a centromere sequence to stabilize the ends of linear eukaryotic chromosomes and to allow for their replication.[10] However, the fact that (i) telomere sequences stabilize the ends of chromosomes and allow for their replication or that (ii) a synthetic C_4A_2 sequence can be used as a telomere in yeast are not at issue. In fact, the application expressly states that use of a $(C_4A_2)_6$ telomere sequence in yeast had already been reported in the art.[11] However, in contrast to conventional procedures, none of the cited references teach or even suggest taking advantage of the short $(C_4A_2)_n$ telomere sequence by placing such sequence into PCR primers that can be used to generate the chromosome splitting vectors (1) and (2) directly as claimed.

Finally, the Office Action states that it would have been obvious to modify the vectors disclosed in **Widianto** with the $(C_4A_2)_n$ telomere sequence in **Ascenzioni** "to provide a

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[8] See *In re Lowry*, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994) ("The Patent and Trademark Office (PTO) must consider all claim limitations when determining patentability of an invention over the prior art.").

[9] See, *e.g., In re Kahn,* 78 U.S.P.Q.2d at 1336. See also, the admonition by the Deputy Commissioner for Patent Operations for the USPTO in a May 3, 2007 memorandum in response to Supreme Court's recent decision in *KSR International Co. v. Teleflex, Inc.*, 127 S. Ct. 1727 (2007) stated that "in formulating a rejection under 35 U.S.C. § 103(a) based upon a combination of prior art elements, it remains necessary to identify the reason why a person of ordinary skill in the art would have combined the prior art elements in the manner claimed."

[10] See, Office Action mailed on June 7, 2007, p. 3 (incorporated by reference); See also Office Action mailed on December 12, 2007, p. 5.

[11] See, Application, p. 3, paragraph [07].

simplified method of splitting the chromosomes" that "would have a reasonable expectation of success, since rearrangement of genetic components...has been routine in the art."[12] However, there does not appear to be any particular advantage gained by using the C_4A_2 sequence of **Ascenzioni** in place of the two opposing telomere sequences in **Widianto** since the difficulty in replicating the repetitive Tr sequences is overcome by using stuffer DNA between the inverted repeats of the Tr sequences.[13] Hence, there would not be a motivation for a person of ordinary skill to combine the cited references since replacing the telomere sequence would not provide any particular advantage other than merely eliminating the step of removing the spacer DNA.

Not only would a person of ordinary skill would have no sufficient reason or motivation to simultaneously view the cited references and replace the Tr telomere sequence in **Widianto** with the $(C_4A_2)_n$ telomere sequence in **Ascenzioni**, but such a combination would still not teach or suggest all of the elements and features of the method as claimed for reasons described above. Therefore, since (i) the combined teachings of the cited references do not teach all of the elements and features of the claimed invention and (ii) a person of ordinary skill would not have reason to combine the cited references in the manner claimed, the rejection of claims 1 and 3 under §103(a) for allegedly being obvious over the cited prior art references is respectfully traversed, and withdrawal of the same is requested. Applicants assert that pending claims 1 and 3 are currently presented in allowable form.

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[12] See, Office Action mailed on December 12, 2007, p. 5.
[13] See, **Widianto**, Materials and Methods, p. 199.

D. CONCLUSION

In view of the foregoing, it is respectfully submitted that this application is in condition for allowance, and favorable action is respectfully solicited. If the Examiner has any questions or concerns regarding the present response, the Examiner is invited to contact Ajay A. Jagtiani at 703-591-2664, Ext. 2001.

The Commission is hereby authorized by this paper to charge any fees during the entire pendency of this application including fees due under 37 C.F.R. §§ 1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 10-0233-NANJ-0009-US1.

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